Ambient Air Asbestos Survey in Quebec Mining Towns
Part 1 — Methodological Study

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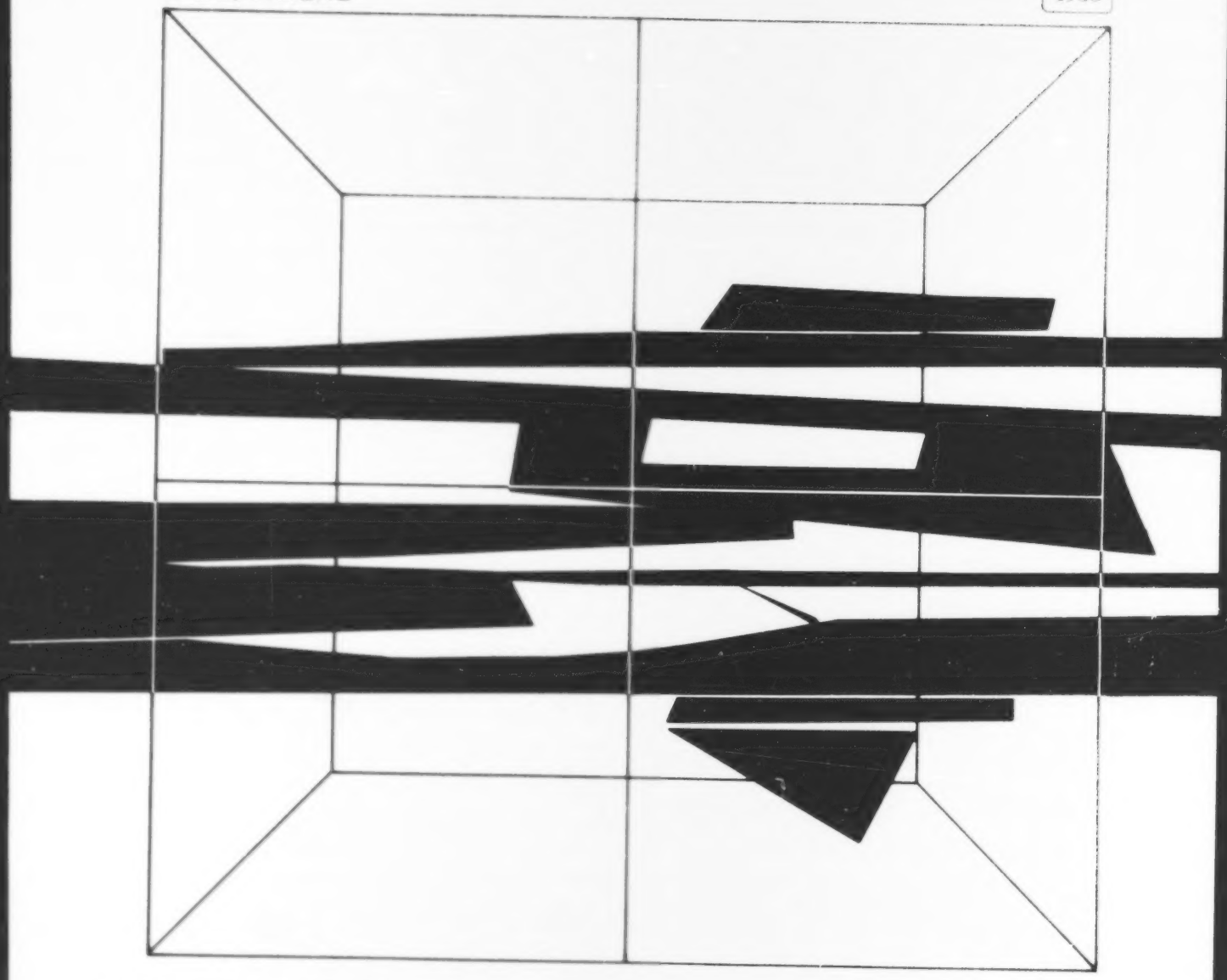

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AMBIENT AIR ASBESTOS SURVEY

IN QUEBEC MINING TOWNS

PART I

METHODOLOGICAL STUDY

by

P. Sébastien, M. Plourde, R. Robb, M. Ross
McGill University

for the

Environmental Protection Service
Environment Canada
Quebec Region

EPS 3/AP/RQ/1E
July 1984

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Minister of Supply and Services
Cat. No. En 49-5/3-RQ1E
ISBN 0-662-93132-7

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ABSTRACT

A methodological study was carried out in order to compare the characteristics of two methods for measuring asbestos air pollution, known as the direct and indirect methods. The two methods are similar in that both make use of analytical electron microscopy (ATEM) to identify, count and measure asbestos particles. They differ in the procedure by which samples are prepared for microscopy. In the direct method, the particles trapped on the sampling surface of a Millipore^{R} membrane filter are "replicated" in a carbon film which is transferred to a ATEM grid. In the indirect method, the membrane is first ashed at low temperature. The ashes are then mechanically agitated by ultrasound in a water bath, redeposited on a Nuclepore^{R} filter, and transferred to ATEM grids.

The main objective of the analysis was to determine the numerical concentration and number-size distribution of chrysotile particles. On the basis of their dimensions, measured directly on the screen, the particles were distributed into a granulometric matrix (15 x 15) which was divided into four blocks (short fibres, "Stanton fibres", "optic fibres", isometric particles). A morphological distinction was made between the fibres, bundles and aggregates.

The two methods were compared by analysing 17 membrane filters taken at Black Lake (Site #736), Thetford Mines (Site #722), and Montreal (Site #012). The sampling instrument was a "Connecticut Lo-Vol", programmed to provide a filtration density of 1 m^3 of air per cm^2 of membrane. Three sampling modes were tested: (a) four hours of sampling per day for two weeks; (b) one day of continuous sampling; (c) four hours of sampling per day for one week.

Sixty-eight ATEM examinations were carried out and 6429 chrysotile particles were counted in total. Although it was possible to analyse all the filters by the indirect method, only six of the membrane filters obtained in the (b) and (c) sampling modes could be analysed by the direct method. These filters were less heavily loaded with particles, owing either to a shorter sampling duration (mode (c)) or to the absence of "passive" sampling (mode (b)). The direct method did not detect significant amounts of asbestos on the two filters taken in Montreal. Comparison of the data obtained from the other four filters, taken in the mining towns and analysed according to one or the other method, revealed a significant "method effect":

(a) The reported concentrations of fine chrysotile particles may be up to 100 times higher with the indirect method. Two possible explanations for this are that these fine particles may be generated by the indirect method and that they are difficult to observe by the direct method.

(b) Because of the protocol for particle counting, the many fine particles monopolize the analytic effort in the indirect method so that the less frequent morphological entities and particle sizes, such as long fibres and chrysotile bundles, tend to be neglected.

Compared with the "method effect", the effect of duration of ultrasound treatment in the indirect method appears negligible. Fine particle concentrations increased by a factor of only three on the average as duration increased from short (one to seven minutes) to long (two hours). Chrysotile bundles appear to be preserved.

Because it is important to have good measurements of levels of pollution by long fibres, it is proposed that an indirect method, modified as follows, be used for the main study:

- filtration density of the Nuclepore^{R} filters will be increased and standardized;

- the duration of ultrasound treatment will be limited to seven minutes;

- only particles longer than 5 microns will be analysed;

- the size of the aliquot for analysis will be increased so as to reach the detection limit of one particle per litre.

RÉSUMÉ

Une étude méthodologique a été effectuée en vue de comparer les méthodes dites "directe" et "indirecte" de mesure de la pollution atmostphérique par l'amiante. Ces deux méthodes sont semblables, en ce sens que toutes deux recourent au microscope électronique à transmission analytique (META) pour identifier, dénombrer et mesurer les particules d'amiante, mais elles diffèrent par la façon dont les échantillons sont préparés avant l'observation au microscope. Selon la méthode directe, les particules retenues sur la face du prélèvement d'une membrane Millipore^R sont "répliquées" dans un film de carbone qui est ensuite transféré sur une grille de META. Par la méthode indirecte, la membrane est incinérée à basse température, puis les cendres sont soumises à une agitation par ultra-sons dans un bain d'eau, et elles sont redéposées sur une membrane Nuclepore^R pour être enfin transférées sur une grille dans un film de carbone.

L'analyse s'est surtout attachée à déterminer la granulométrie en nombre des particules de chrysotile. Sur la base de leurs dimensions mesurées directement sur l'écran, les particules ont été distribuées dans une matrice granulométrique (15 x 15) divisée en quatre blocs (fibres courtes, fibres de Stanton, fibres "optiques", particules isométriques). Une distinction morphologique a été établie entre les fibres, les faisceaux et les agrégats.

Les deux méthodes ont été comparées en analysant 17 membranes provenant de Black Lake (site n^o 736), Thetford Mines (site n^o 722) et Montréal (site n^o 012). L'appareil de prélèvement était un "Connecticut Lo-Vol", programmé pour atteindre une densité de filtration de 1 m^3 d'air par cm^2 de membrane. Trois modes de prélèvement ont été testés : (a) quatre heures de prélèvement par jour pendant deux semaines, (b) une journée de prélèvement continu, (c) quatre heures de prélèvement par jour pendant une semaine.

Au total, 68 analyses par META ont été réalisées et 6429 particules de chrysotile ont été dénombrées au total. Toutes les membranes filtrantes ont pu être analysées par la méthode indirecte, mais seules six membranes utilisées dans les modes (b) et (c) ont pu être analysées par la méthode directe. Ces membranes étaient moins chargées en particules à cause soit d'une durée de prélèvement plus courte [mode (c)], soit par manque de prélèvement "passif" [mode (b)]. La méthode directe n'a pas détecté de quantités importantes d'amiante sur les deux membranes provenant de Montréal. Les

quatre autres membranes venaient des villes minières et la comparaison des résultats obtenus par les deux méthodes a révélé que la méthode d'observation au microscope a un effet important :

(a) les teneurs en fines particules de chrysotile peuvent être jusqu'à 100 fois plus élevées, en utilisant la méthode indirecte. La génération de ces fines particules par la méthode indirecte elle-même et la difficulté que présente leur observation par la méthode directe sont deux explications plausibles de ce phénomène.

(b) Avec la méthode indirecte, lorsqu'on utilise le protocole de numération des particules, les nombreuses particules fines monopolisent l'effort analytique au détriment de l'observation des entités morphologiques et dimensionnelles les moins nombreuses, comme les longues fibres ou les faisceaux de chrysotile.

Comparé à cet effet de la méthode, celui de la durée du traitement aux ultrasons, dans la méthode indirecte, paraît négligeable. En moyenne, entre les temps courts (1 à 7 minutes) et les temps longs (deux heures), les teneurs en fines particules ne sont que trois fois plus élevées lorsque la durée est longue. Les faisceaux de chrysotile semblent ne pas se dissocier.

Étant donné qu'il est important de bien mesurer les niveaux de pollution par les longues fibres, on propose d'utiliser pour l'étude principale, une méthode indirecte modifiée comme suit :

- La densité de filtration sur les filtres Nuclepore^{R} sera augmentée et standardisée.
- La durée de traitement aux ultrasons sera limitée à 7 minutes.
- Seules les particules dont la longueur dépasse 5 microns seront analysées.
- L'aliquote analysée sera augmentée afin d'atteindre la limite de détection d'une particule par litre.

ACKNOWLEDGEMENTS

The investigator wish to express their gratitude to Environment Canada for its financial and organizational support, for the technical help in the collection of air samples, and for the publication of the report. Special thanks to Mrs. Breda Nadon (project engineer) and Mr. Tanny Wypruk (technologist) whose cooperation and expertise were greatly appreciated.

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1 INTRODUCTION

The arguments for carrying out a methodological study prior to the main study were outlined in the initial documents (plenary session, background paper, technical proposal) [1,2]. At that time, the situation could be summarized as follows:

(a) The purpose of the main study was to obtain, over a one-year period, useful data on asbestos air pollution in Black Lake, Thetford Mines, Asbestos, Montreal and Saint Etienne.

(b) It was agreed that the most appropriate method would be transmission electron microscopy (ATEM) of fibres collected on membrane filters [3].

(c) The sampling sites were those sites already used by Environnement Québec.

(d) The sampling instrument was to be the modified "Connecticut Lo-Vol", which had already been acquired and tested by the School of Occupational Health [4].

(e) Two principal procedures called the direct and indirect methods could be envisaged for ATEM examination of the fibres collected on the membrane filters [5,6].

(f) For several reasons, it was decided at the very beginning to use the indirect method to carry out the main study.

(g) No study had been carried out up to that point to compare the results yielded by the two methods. As there were reasons to believe that the results might be different depending on the method, and as a number of laboratories used only the direct or only the indirect type of method, the lack of a comparative methodological study appeared particularly critical.

It was in this context and in order to increase the scope of the main study that the decision was made to undertake a preliminary methodological study comparing the two types of method on the basis of merit, performance and results. Three specific objectives were identified:

(a) To determine the optimal conditions for the indirect method to be used in the main study;

(b) To study the changes in size distribution and initial concentrations occurring during the preparation of membrane filters by the indirect method;

(c) To examine how the total quantity of asbestos was distributed into morphological entities easy to describe microscopically (fibres), and other entities (aggregates, bundles).

It was agreed that, after examination of the report on the methodological study, Environment Canada would inform us of its decision as to the sample analysis protocol for the main study. In this report, we have attempted to present in concise fashion those data from the methodological study which are required for a rapid decision on the protocol for the main study.

2. PROTOCOL FOR THE METHODOLOGICAL STUDY

2.1 Sampling

Sampling begun in mid-January at the nine sites for the main study. A second sampling instrument was set up at three of the sites (Black Lake #736, Thetford Mines #722, Montreal #012) in order to obtain the 18 (six series per site) membrane filters required for the methodological study (see Table I).

The photograph in Figure 1 shows a "Connecticut Lo-Vol" sampling instrument. A metal frame protects the upper rectangular compartment which contains the membrane filter Millipore^R, surface area 400 cm^2, pore size 0,45 micron). A rotary pump with a flow rate of about 110 1/min. is controlled by a time switch, and a temperature compensating gas meter which records the volume of air filtered. The photograph does not show the exhaust system which was installed to eject the filtered air at a respectable distance so as to prevent "turnover", which would lead to underestimation of pollution levels. The "Connecticut Lo-Vol" is capable of operating continuously for four weeks with the same filter. On site, a Magnehelic^R gauge can be used to detect any abnormalities in the pumping line and to determine flow volume instantaneously. The inlet of the "Connecticut Lo-Vol" is the rectangular space between the upper part of the metal frame and the top compartment. To our knowledge, the collection efficiency of such an inlet has never been determined [7].

The initial strategy was to filter about 400m^3 of air through each membrane used in the methodological study in order to attain a filtration density of 1 m^3 air/cm^2 of filter. This is the density felt to be optimal for analysis by the direct method [8]. It is equivalent to approximately 60 hours of sampling by a "Connecticut Lo-Vol" operating at a mean flow volume of 110 1/min., which is a short time compared with the 620 hours (four weeks) of sampling per filter envisaged for the main study. In order to retain the same sampling characteristics, it was decided to use the same flow rate (110 1/min.) for both the methodological and main studies.

First, three consecutive series of filters (see Table I) were obtained at each site. The 60 hours of sampling were distributed over a two-week period by having the instruments operate only four hours a day, from 7:00 to 9:00 A.M. and from 4:00 to 6:00 P.M. A time switch regulating pump operation made it possible to carry out discontinuous sampling in this manner. With this sampling mode, the instruments were not in operation for a high proportion of the time (83%). To explore the possibility of "passive

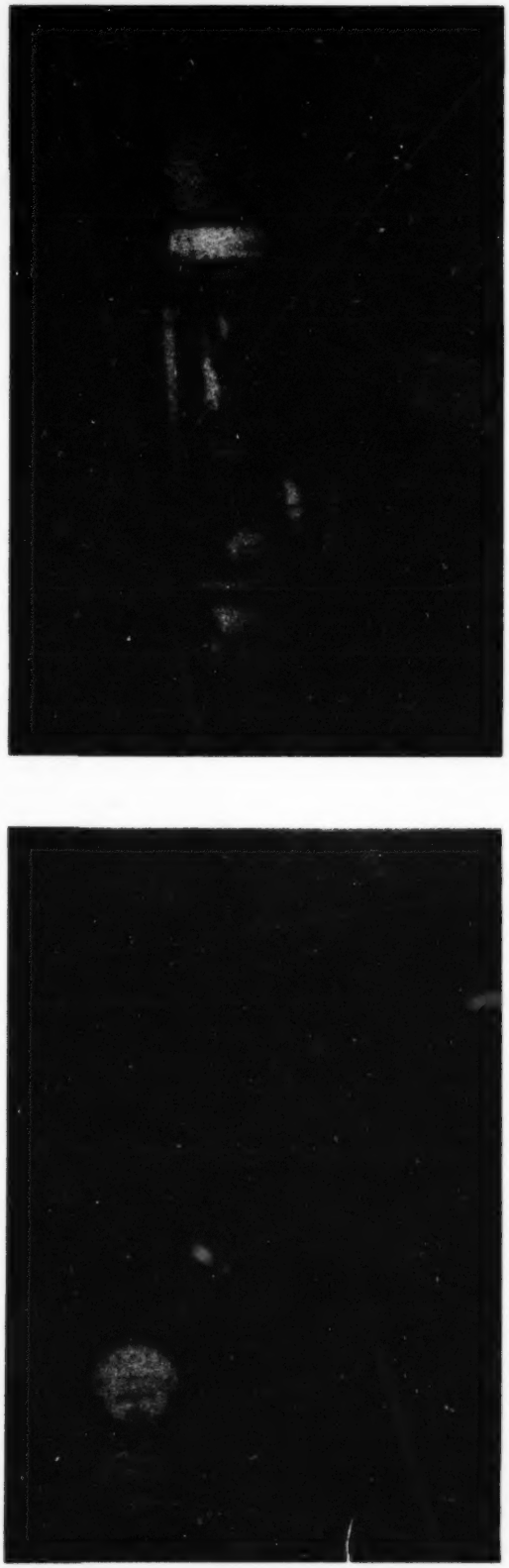


Figure 1. Connecticut Lo-Vol and accessories (Magnehelic^{R} gauge, flowmeter)

sampling" during this dead time, the fourth series of filters was obtained by continuous sampling for one day. For the fifth series, the discontinuous mode (four hours/day) was used again, but over a one-week period only so as to produce less heavily loaded filters. The sixth and final series was obtained by a sampling mode similar to that used for the first three series.

After the improvements the samplers operated in a satisfactory fashion. However, sample collection on filter #21 was defective and it was not possible to analyse it.

2.2 Analysis

The initial protocol provided for joint analysis of each filter by a direct method, two indirect methods, and a gravimetric method (see technical proposal). After the preliminary results, decisions were made to change the protocol. At this point, the first three series of filters were available and had been analysed by the two indirect methods. Unfortunately, it proved impossible to analyse them by the direct method. The filters were too heavily loaded with particles which could not be replicated on a carbon film. During the meeting, a number of important and judicious decisions were made:

(a) Filters which could not be analysed by the direct method would be analysed by an indirect method using short ultrasound treatments. In this way, it was hoped that the initial stages of chrysotile defibrillation could be documented.

(b) A series of filters would be obtained by continuous sampling in order to document the importance of passive sampling.

(c) A series of less heavily loaded filters would be obtained in the hope that the direct method could be used to analyze them.

(d) In the gravimetric method, the phase of chemical treatment of the ash would be excluded from the protocol. Defibrillation would be ensured by a long ultrasound treatment (two hours).

This revised protocol had several attractive aspects. It provided for less disparate analytical methods, and allowed for study on a greater time-scale of the effect of ultrasound duration. It provided the likelihood of obtaining filters that could be analysed by the direct method, an essential

point of the methodological study. It led to the sampling and analysis protocols which are presented in tables I and II and described in the following sections.

2.3 Preparation of membrane filters for analysis by the indirect method

The protocol described in the initial technical proposal was followed. Five pieces measuring 1 cm^2 each were cut from randomly chosen parts of the filter. They were separately placed, sampling side down, on the wall of a cylindrical, wide-mouthed glass tube, which was introduced horizontally into the chamber of a low-temperature asher.

The asher operated throughout the night. In the morning, the vacuum of the asher chambers was broken very gently so that the ash would not be sucked out of the tubes. These were then filled with 100 ml of clean water (Millipore Milli-Q^R), so as to wet the entire surface of the walls. They were then immersed in an ultrasound bath for a set period of time. The physical characteristics of the ultrasound treatment (ultrasound generator, volume of water in bath, type of tube, placement of tubes in bath) were kept constant throughout the study. By means of a probe equipped with a microphone and recording apparatus, it was determined that the suspension was subjected to an ultrasound pressure of 20,000 Pa, with a frequency of 50 kHz.

After the ultrasound treatment, the suspension, or an aliquot, was filtered through a Nuclepore^R membrane (filtration area 10 cm^2, pore size 0.2 micron). The filtration density (m^3 air/cm^2 membrane) was adjusted so as to provide the optimal particle density for microscopic observation. This varied between 0.005 and 0.3 m^3 air/cm^2. Using the conventional replica technique [9], the particles retained on the surface of the Nuclepore^R filter were carbon-coated, and the carbon film transferred to copper ATEM grids (200 mesh, 3 mm diameter). Five grids were prepared, replicating the upper part of the filter in five random locations. The micrograph in Figure 2a illustrates the quality of the preparations thus obtained.

To prepare the filter with a different duration of ultrasound treatment, another five pieces were cut and the entire process was repeated. The filters from series 1, 2, 3 and 6 were prepared according to the above method. For each series, four ultrasound durations were used (see Table II).

TABLE 1 - MEMBRANE FILTERS SAMPLED FOR THE METHODOLOGICAL STUDY

Membrane filter	Sampling mode	Sampling period	Series	Volume of air (m^3)	Mean flow rate (l/min.)
BLACK LAKE (Site #736)					
07	+	Jan. 17 - Jan. 31	1	410.5	110.1
18	+	Jan. 31 - Feb. 14	2	430.3	107.2
27	+	Feb. 14 - Feb. 29	3	475.6	108.6
44	++	Mar. 13 - Mar. 16	4	483.4	104.4
55	+++	Mar. 20 - Mar. 27	5	200.9	105.8
60	+	Mar. 27 - Apr. 10	6	400.2	105.8
THETFORD MINES (Site #722)					
09	+	Jan. 18 - Feb. 1	1	488.4	109.3
21	+	Feb. 1 - Feb. 15	2	Filter damaged	
30	+	Feb. 15 - Mar. 1	3	461.3	108.7
47	++	Mar. 14 - Mar. 16	4	371.6	112.9
56	+++	Mar. 20 - Mar. 28	5	247.3	113.6
61	+	Mar. 28 - Apr. 11	6	425.3	110.8
MONTREAL (Site #012)					
01	+	Jan. 16 - Jan. 30	1	438.8	107.2
14	+	Jan. 30 - Feb. 13	2	424.7	107.9
22	+	Feb. 13 - Feb. 27	3	416.2	112.2
38	+++	Mar. 12 - Mar. 19	5	214.2	110.8
62	+	Mar. 30 - Apr. 12	6	376.9	105.8
63	++	Apr. 16 - Apr. 19	4	472.3	89.48

Sampling mode

\+ Four (4) hours of sampling per day for two weeks
++ One day of continuous sampling
+++ Four (4) hours of sampling per day for one week

TABLE II - ANALYSES PERFORMED

Sampling mode (*)	+	+	+	++	+++	+	+	
Membrane filters								
Black Lake	07	18	27	44	55	60	60	
Thetford Mines	09	-	30	47	56	61	09	
Montréal	01	14	22	63	38	62	-	
Method (**)	Indirect 5' US	Indirect 2' US	Indirect 1' US	Direct	Direct	Indirect 3' US		
	Indirect 15' US	Indirect 15' US	Indirect 10' US	Direct	Direct	Indirect 7' US	Special Indirect 7' US	
	Indirect 30' US	Indirect 30' US	Indirect 20' US			Indirect 50' US	Special Indirect 30' US	
	Indirect 120'US	Indirect 120'US	Indirect 120'US	Indirect 120'US	Indirect 120'US	Indirect 120'US	Special Indirect 120'US	
Number of analyses	12	8	12	9	9	12	6	68

* See Table I for explanation, ** See text for explanation, US: Ultrasound

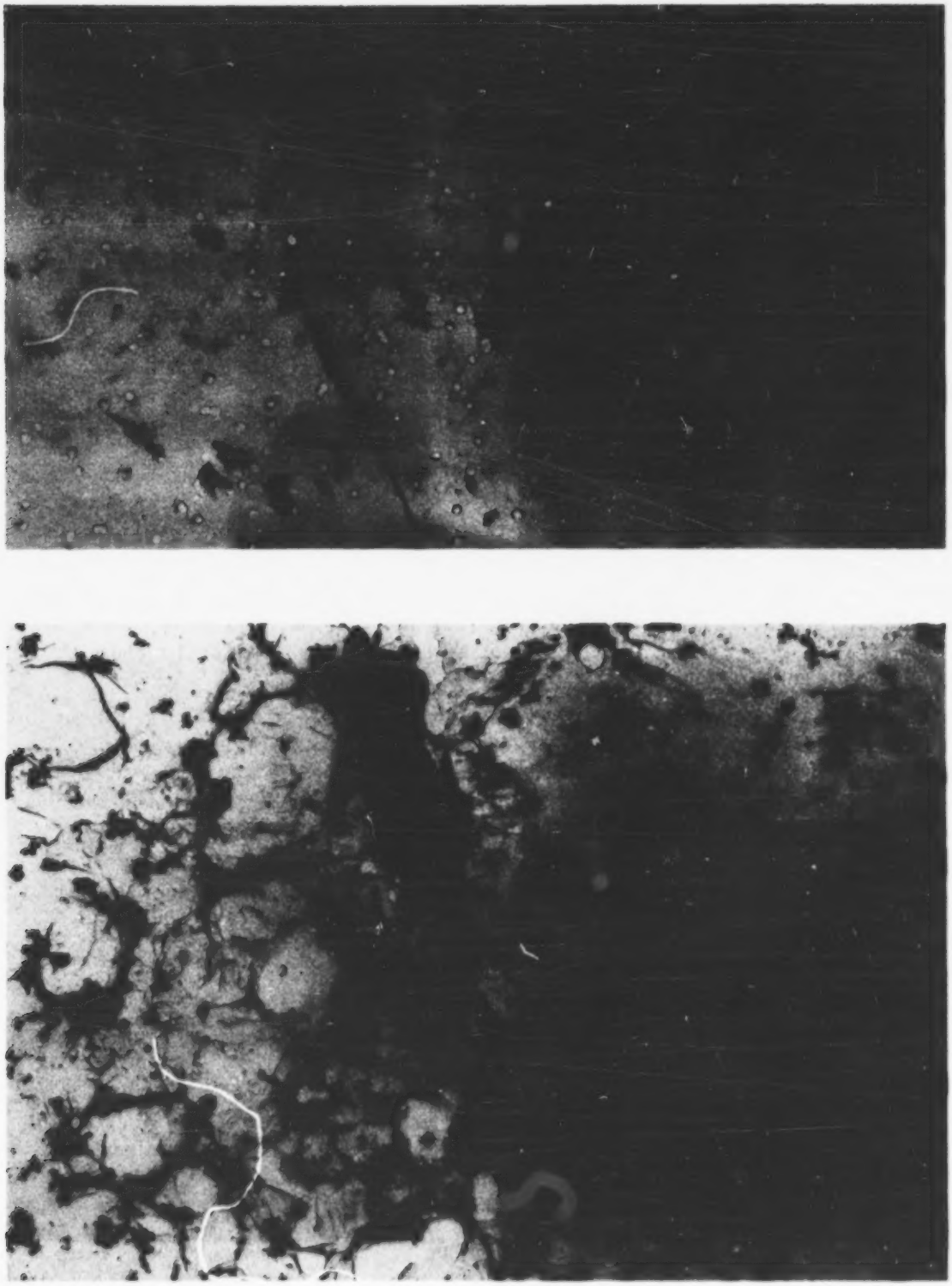


Figure 2. Replica of a Nuclepore^{R} filter in the indirect method (2a) and of a Millipore^{R} filter in the direct method (2b)

2.4 Preparation of membrane filters for analysis by the special indirect method

The method described above introduced two causes of variation in results: the duration of the ultrasound treatment and the heterogeneity of distribution of particles on the filter. In order to eliminate the second, membrane filters #60 and #09 were prepared by filtering aliquots isolated from the same suspension after 7, 30, and 120 minutes of ultrasound on Nuclepore^{R} filters with a filtration area of 2 cm^2, and a pore size of 0.2 micron (see Table II).

2.5 Preparation of membrane filters for analysis by the direct method

With this method, the upper part of the Millipore^{R} filter was directly replicated [10]. First, the thick filter was collapsed by exposing it to acetone vapour in a Petri dish. The purpose of this operation was to make the surface structure of the membrane disappear. The particles could then be replicated on a carbon film, using a technique very similar to that described for the Nuclepore^{R} filters. Figure 2b shows the replicas thus produced. It can be seen that, unlike the replicas of the Nuclepore^{R} filters, these replicas had a filamentous texture which in some cases hindered observation of fine fibrils.

2.6 Analysis of grids

The grids were examined by means of a JEOL 100 CX^{R} ATEM equipped with an energy-dispersive X-ray analyser (EDXA) PGT System IV^{R} for chemical microanalysis. As there was strong reason to believe that the method of membrane filter preparation (direct or indirect) would mostly affect the number of chrysotile particles observed, analysis was directed mainly at the determination of this parameter. A number of grid openings were observed under direct magnification x 16,000 in the transmission mode, with an acceleration voltage of 80 kV. The chrysotile particles were identified either on the basis of their characteristic morphology, or on the basis of their EDXA spectra; little use was made of electron diffraction. The final analysis criteria were: "at least 100 chrysotile particles counted or 14 grid openings observed, whichever comes first".

Each chrysotile particle was classified according to morphological and dimensional criteria. A distinction was made between fibrous morphology, easily described by length and diameter, and other morphologies (aggregates and bundles, see Figure 3). The projected cylindrical dimensions were used to

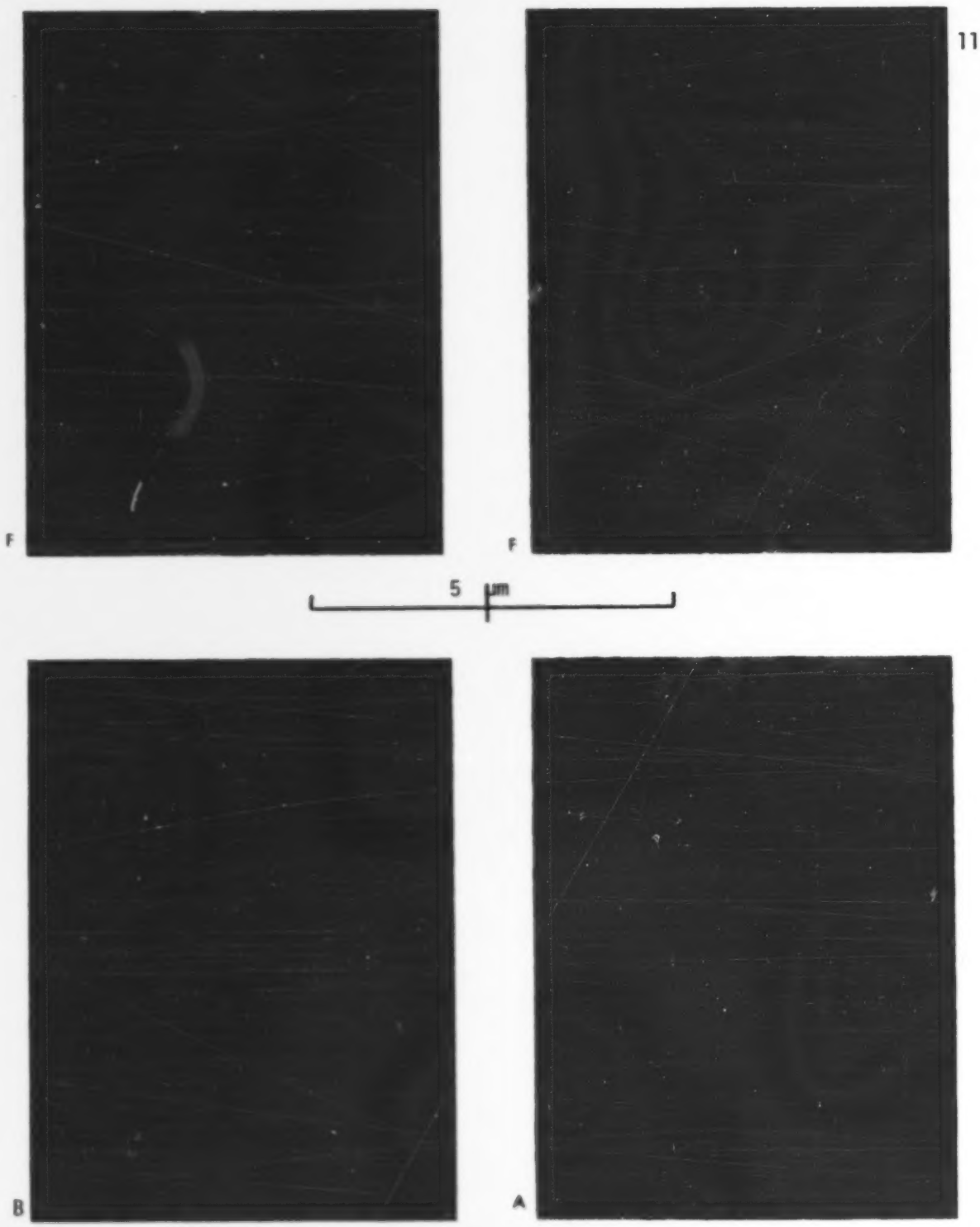


Figure 3 - Classification used for the morphological description of chrysotile particles.

A. Aggregates
B. Bundles
F. Fibers

express the size of bundles and aggregates; the observed particle was placed in an imaginary rectangle, the larger and smaller sides of which were measured and respectively assigned to the length and diameter of the particle. The dimensions were measured directly on the screen, using either a graticule specially designed to measure fibre diameter in 0.03 micron gradations, or a system of two concentric circles 10 and 50 mm in diameter drawn on the screen from which projected dimensions in mm could be estimated (at the magnification being used, 1 mm on the screen would correspond to 0.07 micron).

A computer program was written to process the microscopic data and calculate concentrations, number size distributions, and mass size distributions for each type of particle (e.g., chrysotile fibres, chrysotile aggregates, chrysotile bundles, other fibres).The particles were distributed into a granulometric matrix with a fine division of 225 (15 x 15) size classes and a less fine division of four size classes having preestablished meanings (optic fibres [11], Stanton fibres [12], short fibres, isometric particles; see Figure 4). The output format of the processed microscopic data is reproduced in Table III.

Figure 4

Granulometric matrix allowing distribution of microscopic observations on the basis of particle dimension.

The fine graduation is constituted by 225 (15 x 15) dimensional classes, the limits of which are in geometrical progression.

The matrix is divided into four blocks:

Isometric particles. Particles with a length/diameter ratio of less than 3. Particles with a ratio greater than 3 are called fibres.

Optic fibres. Fibres longer than 5 um, visible under the light microscope. They serve as the basis for industrial health regulations.

Stanton fibres. Fibres with the greatest carcinogenic potential in Stanton's model.

Short fibres. Particles not included in the above three blocks.

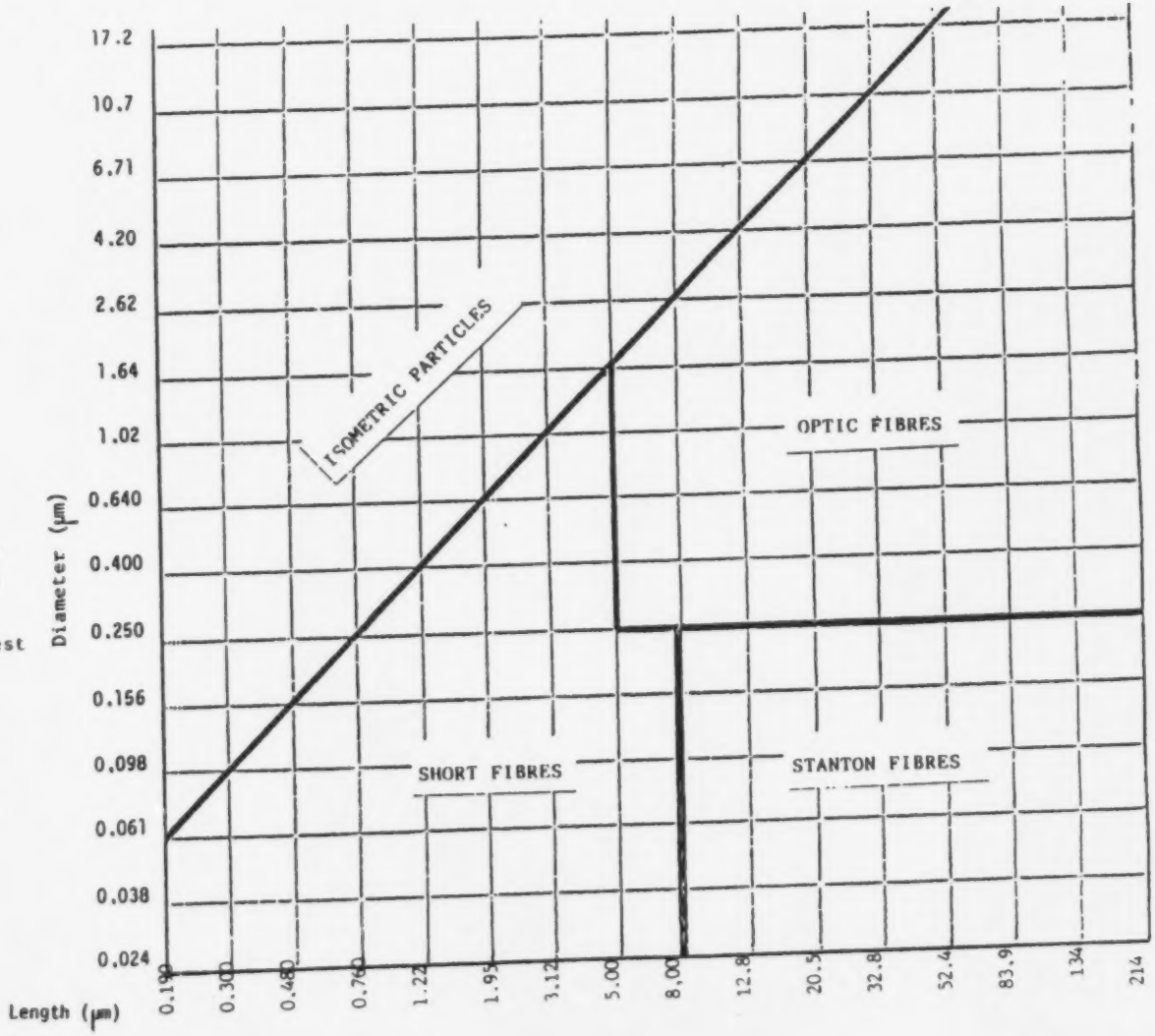


Table 3 - Computerized data

3 RESULTS AND DISCUSSION

As shown in Table II, 68 analyses were performed with a total of 6429 chrysotile particles being identified and sized. The raw data are presented in tables IV, V and VI. The remainder of this document represents an attempt to analyse this mass of data. Emphasis will be placed on the points relevant to the successful completion of the main study.

3.1 Difficulties of the direct method

Unfortunately, the methodological study confirmed initial concerns as to the reliability of the direct method. It proved impossible to prepare the membrane filters of the first three series because their surfaces were so heavily loaded with dust that they could not be replicated on a thin carbon film. However, it was possible to prepare the membrane filters of series 4 (one day of continuous sampling) and 5 (four hours of sampling per day for one week). Although the success of series 5 can be explained by the lower filtration density (approximately 0.5 m^3 of air filtered per cm^2 of filter), an explanation for the success of series 4 is difficult to offer since the filtration density of some of the filters in this series was as high as 1.2 m^3 of air per cm^2 of filter. One would have to postulate that despite their high filtration density, the membrane filters of series 4 in fact had a lower particle load.

This is an interesting point because, since the filters of series 4 were the only ones used for continuous sampling, it raises the possibility that passive sampling occurred in the other series, where the proportion of dead time was high (83%). Support for this hypothesis can be found in the data in Figure 5. For Black Lake and Thetford Mines, a trend was observed toward an increase in pollution over time during the period from mid-January to mid-April. The two levels measured after continuous sampling lie below this curve. The phenomenon is particularly clear for Thetford Mines. It will be seen below that the level reported for the membrane filter from the third series at Black Lake probably constitutes an underestimation, which would fit with the suggested hypothesis. The levels measured in Montreal on membrane filters obtained by discontinuous sampling (four hours per day) were fairly uniform over the period from mid-January to mid-April, while the continuous sampling from April 16 to 19 showed a level three times lower. In these circumstances, it appears that the hypothesis of passive sampling by the "Connecticut Lo-Vol" operating with a dead time of 83% cannot be ruled out. Fortunately, the membrane filters of the main study will be obtained by continuous sampling.

TABLE IV - BLACK LAKE - SITE # 736 - CHRYSOTILE PARTICLES ANALYSED

Series and membrane filter	Ultrasound (minutes)	Aliquot analysed (litres)	Fibre count				Count of other particles				Total, fibres and other particles
			Short	Stanton	Optic	Total	Short	Optic	Isometric	Total	
1 - 07	5	0.0359	72	5		77	13		9	22	99
	15	0.0717	85	2		87	7	1	7	15	102
	30	0.0359	91	1	1	93	8	1	5	14	107
	120	0.0179	106	1		107	1		1	2	109
2 - 18	2	0.0188	81	2		83	11	2	8	21	104
	15	0.0188	155	4		159	1		5	6	165
	30	0.0188	147	7		154	3		5	8	162
	120	0.0188	132			132	13		4	17	149
3 - 27	1	0.0415	130			130	7		7	14	144
	10	0.0208	128			128			1	1	129
	20	0.0208	199	5		204	8	1	16	25	229
	120	0.0164	87	6		93	6		2	8	101
4 - 44	direct	1.1900	25	1	3	29	23	5	7	35	64
	direct	0.6820	56	1		57	4	7	25	36	93
	120	0.0210	122	6		128	3		3	6	134
5 - 55	direct	0.3900	49	2	1	52	15	2	18	35	87
	direct	0.3540	36	3	2	41	4	2	20	26	67
	120	0.0035	109	1		110	2		2	4	114
6 - 60	3	0.0087	119	2		121	11		3	14	135
	7	0.0087	127	4		131	4	1	8	13	144
	50	0.0035	132	2		134	5	1		6	140
	120	0.0035	152	10		162	5	2		7	169
6 - 60	7	0.0035	98	2		100	2		1	3	103
(special	30	0.0035	126	1		127	4		6	10	137
indirect)	120	0.0035	157	3		160	2			2	162

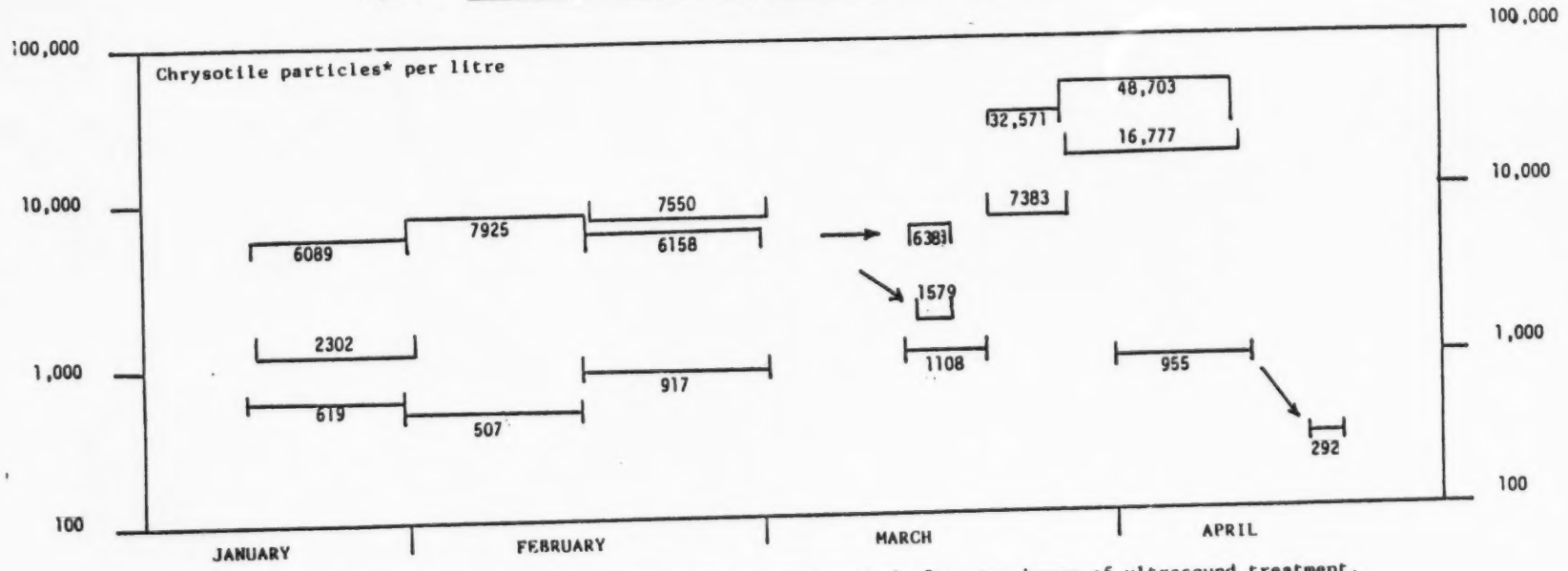
TABLE V - THETFORD MINES - SITE # 722 - CHRYSOTILE PARTICLES ANALYSED

Series and membrane filter	Ultrasound (minutes)	Aliquot analysed (litres)	Fibre count				Count of other particles				Total, fibres and other particles
			Short	Stanton	Optic	Total	Short	Optic	Isometric	Total	
1 - 09	5	0.1070	73	3		76	20		8	28	104
	15	0.1920	86	2		88	6		5	11	99
	30	0.0640	95	1		96	4	1	6	11	107
	120	0.0427	90	5		95			4	4	99
3 - 30	1	0.1000	87	1		88	4	1	7	12	100
	10	0.1000	88			88	4		8	12	100
	20	0.0620	99	1		100	2		2	4	104
	120	0.0201	142	1		143	1		7	8	151
4 - 47	direct	0.9170	7		1	8		2	4	6	14
	direct	0.9170	10		1	11	23	1		24	35
	120	0.0646	96	3		99	2		1	3	102
5 - 56	direct	0.3050	50	1		51	2	4	16	22	73
	direct	0.2620	74	1		75	3	1	11	15	90
	120	0.0214	153	5		158					158
6 - 61	3	0.0555	95	2		97	3	1	2	6	103
	7	0.0185	121	1		122	3		7	10	132
	50	0.0181	123	1		124	6		3	9	133
	120	0.0093	143	2		145	1		5	6	151
1 - 09	7	0.1500	66		1	67	6	1	8	15	82
(special	30	0.0752	90	2		92	4		4	8	100
indirect)	120	0.0430	87	3	1	91	1		1	2	93

TABLE VI - MONTREAL - SITE # 012 - CHRYSOTILE PARTICLES ANALYSED

Series and membrane filter	Ultrasound (minutes)	Aliquot analysed (litres)	Fibre count				Count of other particles				Total, fibres and other particles
			Short	Stanton	Optic	Total	Short	Optic	Isometric	Total	
1 - 01	5	0.2680	14			14	5		3	8	22
	15	0.2680	29			29	6		6	12	41
	30	0.1910	69	1		70	16		13	29	99
	120	0.1340	78			78	2		3	5	83
2 - 14	2	0.2580	40	2		42	2		5	7	49
	15	0.2580	38			38	3		2	5	43
	30	0.2580	41			41	3		2	5	46
	120	0.2030	101			101			1	1	102
3 - 22	1	0.2540	24	1		25	2		2	4	29
	10	0.2540	83			83	1		3	4	87
	20	0.2540	38	1		39	1		1	2	41
	120	0.1090	88	2		90	4		6	10	100
4- 38	direct	0.5290					1			1	1
	direct	0.5290									0
	120	0.0836	90	1		91			1	1	92
5 - 62	3	0.1640	82	1		83	1		2	3	86
	7	0.2300	68			68	3		1	4	72
	50	0.2300	29			29	1			1	30
	120	0.0904	84			84	1			1	85
6 - 63	direct	1.1700									
	direct	1.1700									
	120	0.1440	41			41	1			1	42

Figure 5 CHANGES IN POLLUTION LEVEL OVER TIME AND SAMPLING MODE



* Fibres, bundles and aggregates observed by the indirect method after two hours of ultrasound treatment.

Black Lake, Site #736

Thetford Mines, Site #722

Montréal, Site #012

The arrows indicate the levels measured after continuous sampling over one day. The other levels were measured with membrane filters obtained by discontinuous sampling.

A second difficulty of the direct method relates to the microscopic description of chrysotile particles. To be convinced of these difficulties, one simply has to look at figures 6 and 7 and recall that the purpose of the analysis is to determine the numerical and gravimetric concentration of the particles, as well as the number size and mass size distributions. Can the concept of fibre be used to describe such particles? Can they be described in terms of length and diameter? For Black Lake and Montreal (samples #44, 51, 46 and 57), only 64% of the chrysotile particles observed by the direct method were of isolated fibres for which it was possible to define a true length and diameter. The other chrysotile particles were bundles, or aggregates similar to those shown in figures 6 and 7. They consisted either of agglomerations of chrysotile fibres of complex morphology, or of composite particles in which chrysotile fibres were associated with other types of dust. The use of cylindrical dimensions appeared to be the only way of codifying these observations systematically. However, such a system of codification, which ignores the morphological variety of the particles, leads to an over-simplification of the situation.

The indirect method proved almost useless in Montreal, because in the four analyses, only five chrysotile particles were found, which is too low a number for the calculation of concentrations. In Montreal, no particles of chrysotile were encountered similar to those of Black Lake or Thetford Mines, whose size could reach 30 to 50 microns. In our view, the presence of particles such as those illustrated in figures 6 and 7 constitutes the specific and significant component of asbestos air pollution in mining towns.

3.2 Comparison of the direct and indirect methods

Which method gives the more accurate estimate of the true morphological and dimensional characteristics of the dust particles? Joint analysis by both the direct method and an indirect method (two hours of ultrasound treatment) of four membrane filters (#44, 47, 55 and 56) from Black Lake and Thetford Mines unfortunately showed very large differences in the results yielded by the two methods:

(a) Reported chrysotile concentrations can be up to 150 times higher with the indirect method.

(b) The two methods yield different morphological and dimensional distributions of the particles observed.

Figure 6. Examples of chrysotile particles encountered on membrane filters from Black Lake and Thetford Mines analysed by the direct method

FIGURE 7 - Examples of chrysotile particles encountered on membrane filters from Black Lake and Thetford Mines analysed by

The data supporting these two statements are presented in tables VII and VIII. Table VII shows the mean numerical concentrations for the four filters, distributed into the 225 dimensional classes of Figure 4. The format of this table clearly illustrates the marked differences in particle number as measured by the two methods. With the indirect method, significant crowding is observed toward the lower part of the granulometric matrix, and this is accompanied by a very large increase (mean of about 50X) in numerical concentrations.

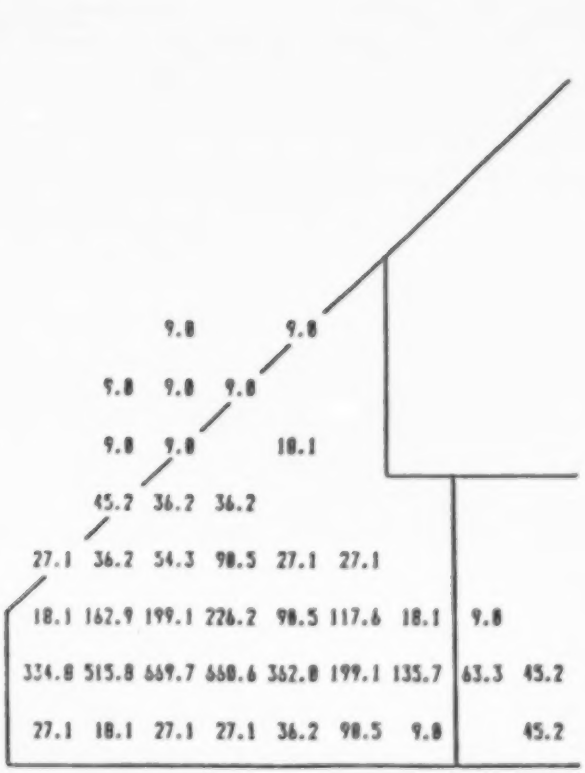
With very rare exceptions (see Figure 8), the bundles and aggregates of the type illustrated in figures 6 and 7 are not encountered with the indirect method. Most of the microscopic observations made with the indirect method led to the classification "short fibre". For the four membrane filters under consideration, "fibres" accounted for over 95% of the microscopic observations made by the indirect method after two hours of ultrasound treatment. These were short fibres for the most part; there were no optic fibres, and Stanton fibres accounted for only 3% of the total. It may be that if ultrasound treatment had been shorter, the percentage of fibres would have been less. However, as can be seen from Table IX, even with short ultrasound times, the percentage of particles classified as fibres remains high, in the neighbourhood of 85%.

With the direct method, on the other hand, the proportion of particles classified as "fibres" could be as low as 34% (see Table VIII), with the balance consisting of bundles and aggregates much larger than those observed with the indirect method (see Table VII).

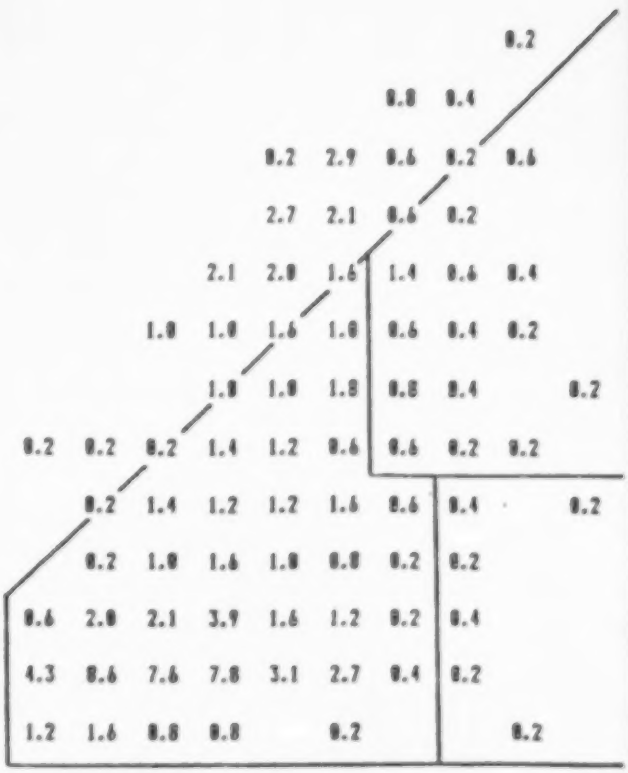
What accounts for these differences in the microscopic observations made by the two methods in the analysis of the membrane filters from Black Lake and Thetford Mines? Figure 9, which shows the distribution of numerical concentrations for several classes of diameter, gives another illustration of these differences. The distribution given by the direct method does not have a simple shape. In particular, there is a bulge at the large-diameter end, certainly caused by the observation of large particles, but also probably by the use of cylindrical coordinates. The curve for the indirect method shows much higher numerical concentrations and has a more regular shape; the observed diameters do not exceed 1 micron, and in this area the numerical concentrations approach the limit of detection.

In an attempt to explain the data in Figure 9, one can imagine the following scenario. Initially, incineration liberates fine fibres which have coagulated onto organic particles or penetrated within the structure of the

TABLE VII - COMPARISON OF DIRECT AND INDIRECT METHODS
NUMERICAL CONCENTRATIONS (PARTICLES*/LITRE) IN THE CLASSES



Indirect method
(2 hours of ultrasound treatment)



Direct method

Cumulative observations, membrane filters #44 + 55 + 47 + 56

* Including chrysotile fibres, bundles and aggregates

TABLE VIII MICROSCOPIC OBSERVATIONS WITH THE TWO METHODS

	Aliquot analysed		Particles*/litre		Proportion of fibres (%)	
Membrane filter	Direct method	Indirect method**	Direct method	Indirect method**	Direct method	Indirect method**
44	1,872	0.021	84	6.381	55	95
55	0,744	0.003	207	32.571	60	96
47	1,834	0.065	16	1.579	34	97
56	0,667	0.021	244	7,383	77	100

* Including chrysotile fibres, bundles and aggregates

**Two hours of ultrasound treatment

TABLE IX - INDIRECT METHOD - EFFECT OF ULTRASOUND TREATMENT ON NATURE OF MICROSCOPIC OBSERVATIONS*
Percentage of observations classed as "fibres"

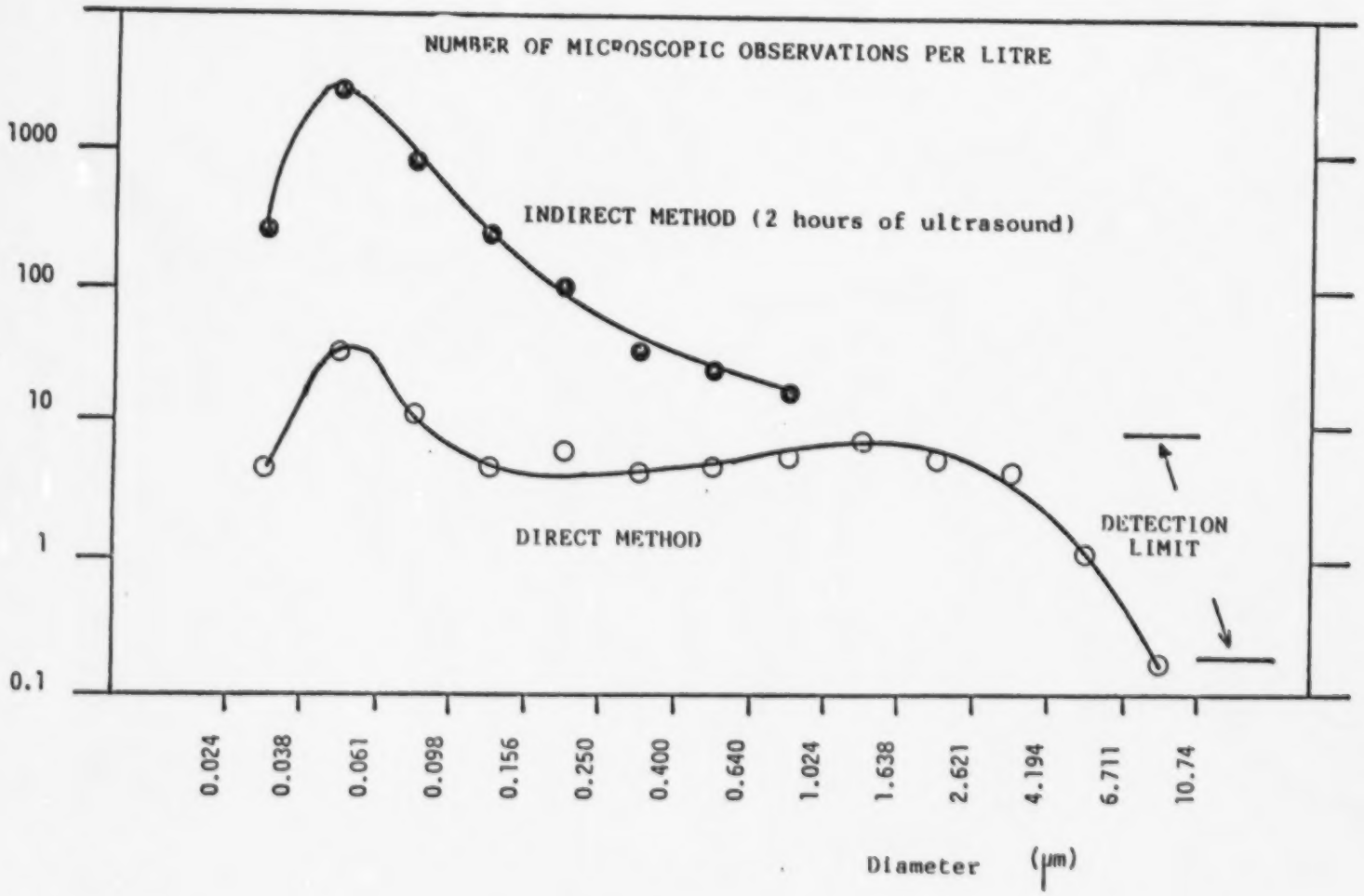
Filter membrane	Duration of ultrasound treatment (minutes)										
	1	2	3	5	7	10	15	20	30	50	120
07				77.7			85.3		86.9		98.2
18		79.8					94.4		95.0		88.6
27	90.3					99.2		94.3			92.1
44											95.3
55											96.5
60			89.6		90.9					95.7	95.9
60 Special					97.1				92.7		98.7
09				73.1			88.9		89.7		96.9
30	88.0					88.0		96.1			94.7
47											97.0
56											100.0
61			94.2		92.4					93.2	96.0
09 Special					81.7				92.0		97.8
Mean (standard deviation)			86.8 (7.6)					92.2 (3.9)			95.9 (2.9)

* Concerns chrysotile only.

FIGURE 8 - Indirect method - Chrysotile bundle persisting after ultrasound treatment for 7 minutes

Figure 9. COMPARISON OF DIRECT METHOD AND INDIRECT METHOD*

DISTRIBUTION OF DIAMETERS



* Including chrysotile fibres, bundles and aggregates.

membrane filter. Then, aqueous suspension and ultrasound treatment results in the release of fibres from bundles and aggregates. These three events together (incineration, suspension, ultrasound treatment) increase the number of fine fibres so that they become the most numerous. Since the analysis ends after 100 microscopic observations, the most numerous type of fibre is that with the highest likelihood of being taken into account by the analysis. The latter thus tends to ignore less numerous entities, such as bundles or aggregates which have lost some of their fibres, but which persist even after ultrasound treatment. Thus, the production of an abundance of short and fine fibres could explain both the increase in the number of microscopic observations and the failure to observe large particles with the indirect method.

The hypothesis regarding the production of the short fibres observed by the indirect method is confirmed by the data in Table X. It can be seen that this is a significant phenomenon, the extent of which varies between samples, but which leads on the average to an increase in numerical concentration by a factor of 50. As will be seen below, compared with this effect, the effect of duration of ultrasound treatment is negligible.

At this stage, it is impossible to state whether bundles and aggregates larger then 1 micron in diameter persist after ultrasound treatment. If they do persist, in order to observe them correctly it would be necessary to lower the detection limit of the indirect method by a factor of 10, that is, terminate analysis after 1000 microscopic observations, which would be almost impossible to do. However, this point could be documented by combining the observations carried out on all the membrane filters analysed by the indirect method. Since the total number of observations is close to 5000, overall detection should be sufficient for accurate description. This work has yet to be done for all 225 dimensional classes. It would lead to a representation similar to that in Table VII. By way of illustration, the compilation was carried out for the optic fibre category, an interesting one because it contains large particles. The data are reported in Table XI, which illustrates two essential points:

(a) It confirms that the detection threshold of the indirect method is much higher than the detection threshold of the direct method. After 38 analyses using the indirect method for samples taken at Black Lake and Thetford Mines and 4752 microscopic observations, the total aliquot analysed was only 1.5357 litres. However, after only 16 analyses using the direct method and 503 microscopic observations, the total aliquot analysed was 5.0170 litres.

TABLE X - NUMERICAL CONCENTRATIONS (FIBRES/LITRE) OF SHORT CHRYSOTILE FIBRES OBSERVED BY THE TWO METHODS

Membrane filter	Direct method	Indirect method*
44	43	5809
55	114	31142
47	9	1477
56	218	7286

* With two hours of ultrasound treatment

(b) It shows that if the detection threshold of the indirect method is lowered, it becomes possible to describe relatively less numerous entities, such as large optic fibres, and to obtain numerical concentrations for this type of entity which are very close to those measured by the direct method.

The previously described scenario suggested that the direct method gives a closer representation of the truth, and attempted to explain how the protocol for the indirect method leads to a distortion of initial concentrations and size distributions of particles. A second scenario can be envisaged which, conversely, would portray the indirect method as giving a closer representation of the truth and would expose observational artefacts in the direct method. With such a working hypothesis, the data in Figure 9 would be interpreted as an underestimation of fine particles with the direct method. The micrograph in Figure 2b, together with other factors, provides several arguments in favour of this hypothesis:

(a) An important "masking" effect often occurs with the direct method, due to the presence of large particles on the membrane filter.

(b) The replica of the Millipore^{R} membrane filter has a texture which complicates observation and can lead to underestimation of fine particles.

(c) Apparently some parts of the membrane filter are not replicated or are poorly replicated, producing spaces in the film which contain no particles.

(d) Fine particles can penetrate into the membrane filter during sampling. These are not "recovered" by the carbon film, which replicates only the surface of the filter.

As it is difficult to quantify these phenomena, it is difficult to say whether they are of sufficient extent to explain the differences in the numerical concentrations of fine particles reported by the two methods. It should be noted, however, that the combination of the two phenomena - production of fine particles by the indirect method and underestimation of fine particles by the direct method - probably accounts for the differences in the numerical concentrations of fine particles reported by the two methods.

This scenario cannot be extended to apply to large particles, which are revealed only by the direct method. As was seen above, statistical considerations relating to detection limits (see Table XI) may explain the apparent absence of large particles on individual membrane filters analysed by the indirect method.

It should be remembered that the hypotheses developed in this section relate only to samples in the mining towns, because in Montreal the direct method did not yield enough microscopic observations.

3.3 Indirect method: effect of duration of ultrasound treatment

Tables IX, XII and XIII show that, on the average, reported numerical concentrations increased with duration of ultrasound treatment. The increase was mainly in the number of short fibrils.

In order to simulate the analysis conditions of the main study, the first series of experiments used distinct lots of five pieces cut from the membrane filter for each treatment duration. Under these conditions, even if the main values of Table XIII tend to increase, the trend is not systematic. To observe a systematic increase, the heterogeneity of distribution of fibres on the filter surface would have to be eliminated, and the membrane filters would have to be prepared by the method referred to as the "special indirect method". Note that the main increase in numerical concentrations between short treatment times (up to seven minutes) and longer periods (two hours) is only in the order of three times. This must be compared with the 50 times difference between the direct method and the indirect method with two hours of ultrasound treatment.

In Montreal, the increase was much less marked, indeed, hardly perceptible.

We have already seen that ultrasound can liberate short fibrils. Does ultrasound cause defibrillation of long bundles of chrysotile? The data reported in Table XIV indicate that this is doubtful. Since there is, generally speaking, a positive relationship between bundle diameter and length, long bundles contain many fibres and their defibrillation should produce a large number of finer fibres, such as "Stanton fibres". The proportion of these should increase significantly with the duration of ultrasound treatment, but this is not the case. This observation is important insofar as it tends to demonstrate that the indirect method preserves large chrysotile particles.

TABLEAU XI. DETECTION LIMIT AND NATURE OF MICROSCOPIC OBSERVATIONS

Membrane filter	Indirect method*			Direct method		
	Aliquot analysed (litres)	Microscopic observations	Optic fibres	Aliquot analysed (litres)	Microscopic observations	Optic fibres
07	0.1614	417	3			
18	0.0752	580	2			
27	0.0995	603	1			
44	0.0210	134	0	1.872	157	15
55	0.0035	114	0	0.744	154	7
60	0.0244	588	1			
60 Special	0.0105	402	0			
09	0.4060	409	1			
30	0.2800	455	1			
47	0.0650	102	0	1.834	29	5
56	0.0210	158	0	0.567	163	6
61	0.1000	519	1			
09 Special	0.2682	271	3			
Total	1.5357	4752	13 (8,5/Litre)	5.017	503	33 (6,6/Litre)

* All durations of ultrasound treatment considered together.

** Including chrysotile fibres, bundles and aggregates.

TABLE XII - INDIRECT METHOD - EFFECT OF DURATION OF ULTRASOUND TREATMENT ON THE NUMERICAL CONCENTRATION (PER LITRE) OF MICROSCOPICALLY OBSERVED PARTICLES*

Membrane filter	Duration of ultrasound treatment (minutes)										
	1	2	3	5	7	10	15	20	30	50	120
07				2757			1422		2980		6089
18		5532					8776		8617		7925
27	3470					6202		11009			6158
60			15482		16514					40346	48703
60 Special					29428				39142		46286
09				972			516		1672		2302
09 Special					520				1330		2163
30	1000					1000		1733			7550
61			1873		7333					7389	16777
01				82			153		518		619
14		190					167		178		502
22	114					342		161			917
62			524		313					130	944

* Including chrysotile fibres, bundles and aggregates.

TABLE XIII - INDIRECT METHOD. EFFECT OF DURATION OF ULTRASOUND TREATMENT ON NUMERICAL CONCENTRATION OF MICROSCOPICALLY OBSERVED PARTICLES*. EXPRESSION OF RATIO (CONCENTRATION AT DURATION T)/(CONCENTRATION AT DURATION TWO HOURS)

Membrane filter	Duration of ultrasound treatment (minutes)										
	1	2	3	5	7	10	15	20	30	50	120
07				0.45			0.23		0.49		1.00
18		0.69					1.10		1.01		1.00
27	0.56					1.00		1.78			1.00
60			0.32		0.34					0.83	1.00
60 Special					0.63				0.85		1.00
09				0.42			0.22		0.73		1.00
09 Special					0.24				0.61		1.00
30	0.13					0.13		0.23			1.00
61			0.11		0.44					0.44	1.00
01				0.13			0.25		0.84		1.00
11		0.38					0.33		0.35		1.00
22	0.12					0.37		0.17			1.00
62			0.55		0.33					0.14	1.00
Mean (standard diviation)		0.365 (0.186)						0.576 (0.420)			

* Including chrysotile fibres, bundles and aggregates.

TABLE XIV - INDIRECT METHOD. EFFECT OF ULTRASOUND ON NATURE OF MICROSCOPIC OBSERVATIONS*. PERCENTAGE OF OBSERVED PARTICLES CLASSIFIED AS STANTON FIBRES.

Membrane filter	Duration of ultrasound treatment (minutes)										
	1	2	3	5	7	10	15	20	30	50	120
07				6.5			2.3		1.07		0.9
18		2.4					2.5		4.5		0.0
27	0.0					0.0		2.5			6.5
44											4.7
55											0.0
60			1.6		3.0					1.5	5.9
60 Special					2.0				0.8		1.9
09				3.9			2.3		1.0		5.3
09 Special					0.0				2.2		3.3
30	1.1					0.0		1.0			0.7
47											3.0
56											3.2
61			2.0		0.8					0.8	1.4
Mean (Standard deviation)			2.12 (1.87)					1.60 (1.20)			2.83 (2.23)

* Including chrysotile fibres, bundles and aggregates.

4 CONCLUSIONS AND RECOMMENDATIONS

Although the aim of this study was to cover solely the methodological aspects, a number of observations of a different nature are worthy of mention:

(a) Pollution increased over time in the mining towns but not in Montreal.

(b) Pollution was higher in Black Lake than in Thetford Mines.

(c) Large particles of chrysotile were present exclusively in the mining towns.

With respect to methodology, despite the small number of membrane filters analysed by both methods, the comparative characteristics of the two methods are perceived as follows:

Direct method:

(a) Low reliability because of uncertainty of successful preparation of the replica.

(b) Better detection limit than the indirect method, allowing observation of a larger aliquot and, as a result, description of less frequently occurring morphological and dimensional entities.

(c) May underestimate fine particles.

(d) Does not permit asbestos and other associated particles to be studied separately, necessitating use of cylindrical dimensions, which are not very specific.

(e) Apparently not usable in Montreal, which is a very critical factor as it deprives the study of comparative data.

Indirect method:

(a) Reliable.

(b) Provides quality replicas on which asbestos particles are presented in a format compatible with microscopic description.

(c) Definitely leads to overestimation of fine particle concentrations.

(d) The abundance of fine chrysotile fibrils gives the method a high detection limit with respect to the less frequent morphological and dimensional entities.

This study does not conclude that duration of ultrasound treatment has a determining effect on numerical concentrations and the nature of microscopic observations. Two hours of ultrasound treatment can increase numerical concentrations of fine fibres by a factor of about three. This effect, which can be masked by heterogeneity in particle location on the membrane filter, is low compared with the "method" effect caused by changing from the direct to the indirect method. As has been shown by others [13], short ultrasound treatment durations have little effect on size distribution of chrysotile particles.

The differences in the results yielded by the two methods can be explained as follows. Because of the structure of chrysotile, any sample of chrysotile dust will naturally contain a high proportion of fine particles. The direct method tends to underestimate the number of these particles, either because they have coagulated onto other particles, or because they have not been replicated on the carbon film. On the other hand, the large particles, which are less subject to the masking effect and are better replicated even though they are less frequent, are well demonstrated by the direct method, which has a good detection limit.

During preparation of the membrane filters in the indirect method, a large number of fine chrysotile particles (particles which have coagulated onto organic matter, or become detached from chrysotile bundles or other dusts) are generated. Because of the counting protocol, these fine particles monopolize the analytic effort to the detriment of the observation of less frequent morphological and dimensional entities, such as long, wide fibres and chrysotile bundles. The latter seem not to be destroyed by ultrasound treatment, even though they do lose some of their fine, loosely bound fibrils. However, owing to biological considerations, it may be important to analyse these entities correctly [14].

Given these conditions, we propose that the main study be carried out with a lower detection limit for the indirect method, so as to analyse long fibres correctly. In order to do this, the following changes will be made to the protocol for the indirect method [2]:

- Filtration density on Nuclepore^{R} filters will be increased and standardized.

- The duration of ultrasound treatment will be limited to seven minutes.

- The magnification at which the preparations are observed will be reduced to 10,000.

- Analysis will be limited to particles longer than 5 microns.

- The number of openings analysed will be increased in order to attain the detection limit of one particle per litre.

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